THE JOURNAL OF BIOLOGICAL CHEMISTRY

Vol. 264, No. 10, Issue of April 5, pp. 5593-5597, 1989
Printed in U.S.A.

Dehydrogenase-dependent Ethanol Metabolism in Deer Mice (*Peromyscus maniculatus*) Lacking Cytosolic Alcohol Dehydrogenase

REVERSIBILITY AND ISOTOPE EFFECTS *IN VIVO* AND IN SUBCELLULAR FRACTIONS*

(Received for publication, September 7, 1988)

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Elimination of [^{2}H]ethanol *in vivo* as studied by gas chromatography/mass spectrometry occurred at about half the rate in deer mice reported to lack alcohol dehydrogenase (ADH$^-$) compared with ADH$^+$ deer mice and exhibited kinetic isotope effects on V_{max} and K_m ($^D(V/K)$) of 2.2 ± 0.1 and 3.2 ± 0.8 in the two strains, respectively. To an equal extent in both strains, ethanol elimination was accompanied by an ethanol-acetaldehyde exchange with an intermolecular transfer of hydrogen atoms, indicating the occurrence of dehydrogenase activity. This exchange was also observed in perfused deer mouse livers. Based on calculations it was estimated that at least 50% of ethanol elimination in ADH$^-$ deer mice was caused by the action of dehydrogenase systems.

NADPH-supported cytochrome P-450-dependent ethanol oxidation in liver microsomes from ADH$^+$ and ADH$^-$ deer mice was not stereoselective and occurred with a $^D(V/K)$ of 3.6. The $^D(V/K)$ value of catalase-dependent oxidation was 1.8, whereas a kinetic isotope effect of cytosolic ADH in the ADH$^+$ strain was 3.2. Mitochondria from both ADH$^+$ and ADH$^-$ deer mice catalyzed NAD$^+$-dependent ethanol oxidation and NADH-dependent acetaldehyde reduction. The kinetic isotope effects of NAD$^+$-dependent ethanol oxidation in the mitochondrial fraction from ADH$^+$ and ADH$^-$ deer mice were 2.0 ± 0.1 and 2.3 ± 0.3, respectively. The results indicate only a minor contribution by cytochrome P-450 to ethanol elimination, whereas the isotope effects are consistent with ethanol oxidation by the catalase-H_2O_2 system in ADH$^-$ deer mice in addition to the dehydrogenase systems.

Ethanol elimination is catalyzed predominantly via metabolism in the liver. Alcohol dehydrogenase (ADH)[1] enzymes (EC 1.1.1.1) present in the cytosolic fraction of the liver (*cf.* Refs. 1 and 2) are believed to be responsible for the majority of ethanol oxidation, whereas the contribution of non-ADH systems including catalase and cytochrome P-450 is currently under debate (*cf.* Refs. 3–5).

Several attempts to evaluate the role of non-ADH pathways in ethanol oxidation have been performed using two strains of deer mice (*Peromyscus maniculatus*), one containing cytosolic ADH (Adh^F/Adh^F) (ADH$^+$) and one reported to lack ADH (Adh^N/Adh^N) (ADH$^-$) (6, 7). Experiments have been carried out *in vivo* and *in vitro* with inhibitors of catalase and cytochrome P-450 known to be nonspecific (8–14) such as azide, aminotriazole, butanol, and 4-methylpyrazole (4-MP).

We have recently evaluated isotope effects of cytochrome P-450-dependent ethanol oxidation in microsomal fractions of rat liver in the presence of NADPH (15). Kinetic isotope effects ($^D(V/K)$) of about 4 and lack of stereoselectivity were observed for ethanol oxidation in microsomes or reconstituted vesicles containing the ethanol-inducible form of rabbit liver microsomal cytochrome P-450 (*cf.* Refs. 16–18). Thus, the isotope effects and stereoselectivity differed from those of other ethanol-oxidizing systems and could provide a basis for examination of the relative contributions of these enzyme systems to ethanol elimination under *in vivo* conditions or in perfused liver systems. Changes in the composition of mixtures of deuterated ethanols may be used to calculate isotope effects and in addition the extent of reversibility and concomitant intermolecular hydrogen transfer (*cf.* Fig. 1) (19). Thus, in order to differentiate between the contributions of ADH and non-ADH enzyme systems to ethanol oxidation, advantage can be taken of the reversibility *in vivo* that only occurs with dehydrogenases (19–21). We have now applied this experimental system to deer mice in order to evaluate the relative contributions of various alcohol-metabolizing systems to ethanol elimination *in vivo*.

EXPERIMENTAL PROCEDURES

Materials

[1,1-$^2H_2$]Ethanol (99.6% ^{2}H), [2,2,2-$^2H_3$]ethanol (99.0% ^{2}H), [1,1,2,2,2-$^2H_5$]ethanol (99.2% ^{2}H), and [$^2H_4$]acetaldehyde (99.5% ^{2}H) were obtained from Alfred Hempel GmbH & Co. (Düsseldorf, Federal Republic of Germany), [$^2H_6$]ethanol (99% ^{2}H) from Merck AG (Darmstadt, FRG), and [1-^{13}C]ethanol (90% ^{13}C) from Merck Sharp and Dohme. (1*R*)- and (1*S*)-[1-^{2}H]ethanol were prepared and purified as described previously (15, 22). The isotopic composition of the alcohols used was determined by GC/MS of the 3,5-dinitrobenzoates (19). NADPH, NADH, NADP$^+$, NAD$^+$, phenylmethylsulfonyl fluoride, and 4-MP were purchased from Sigma, and EDTA was from Fluka (Buchs, Switzerland). Glucose oxidase (19,500 units/g) was from Boehringer Mannheim. Analytical grade dimethylformamide obtained from BDH Chemicals Ltd. (Poole, England) was distilled over 2,4-dinitrophenylhydrazine prior to use. Dinitrophenylhydrazine and 3,5-dinitrobenzoyl chloride were obtained from Merck AG and re-

* This work was supported by Grant AA-03624 from the National Institute on Alcohol Abuse and Alcoholism, Grant ARA 8512 from the North Carolina Alcoholism Research Authority, and Grants 2189 and 5949 from the Swedish Medical Research Council. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] The abbreviations used are: ADH, alcohol dehydrogenase; ADH$^-$, deer mice reported to be genetically deficient in alcohol dehydrogenase; ADH$^+$, deer mice having alcohol dehydrogenase; GC/MS, gas chromatography/mass spectrometry; $^D(V/K)$, kinetic isotope effect on V_{max}/K_m; 4-MP, 4-methylpyrazole.

crystallized from ethanol and benzene, respectively. Desferrioxamine was from Ciba-Geigy (Basel, Switzerland), sodium azide and semicarbazide from Merck AG, and calcium cyanamide from American Cyanamide Co. (Wayne, NJ).

Animals

Two strains of deer mice (*P. maniculatus*), one with reported normal levels of hepatic ADH (Adh^F/Adh^F) (ADH^+) and one reported to lack ADH (Adh^N/Adh^N) (ADH^-), were obtained from a breeding colony at the University of North Carolina at Chapel Hill. These animals were established from breeding pairs kindly supplied by Dr. M. R. Felder of the University of South Carolina. The deer mice were fed chow diet and water *ad libitum* and housed in rooms with a 16-h light and 8-h dark cycle.

Methods

In Vivo Experiments—A 1:1 mixture of [1,1-$^2H_2$]- and [2,2,2-$^2H_3$]ethanol (2 g/kg) was injected intraperitoneally into female deer mice. Blood samples (20 μl) were taken from the retro-orbital sinus at 30-min intervals and added to 0.2 ml of 1 M perchloric acid containing [$^2H_6$]ethanol as the internal standard. After neutralization with 1 M KOH, 3,5-dinitrobenzoate esters of ethanol were prepared essentially as described previously (19) and analyzed by GC/MS (21). Similar experiments were performed with 20 mM [^{2}H]ethanol mixtures in recirculating liver perfusion systems as described previously (9).

Preparation of Subcellular Fractions—Microsomes were prepared by differential centrifugation from livers homogenized in 4 volumes of 1.14% KCl and were suspended in 50 mM potassium phosphate buffer, pH 7.4, yielding a protein concentration of about 25 mg/ml. Mitochondrial fractions were prepared from livers homogenized in 0.25 M sucrose containing 0.25 mM phenylmethylsulfonyl fluoride. The mitochondria were washed twice in 0.25 M sucrose containing 10 mM Tris-HCl, pH 7.4, suspended in the incubation buffer, and treated with 0.08% Triton X-100 for 15 min prior to incubation.

Measurements of in Vitro $^D(V/K)$ and Stereoselectivity—Incubations with ethanol were carried out using 25-ml stoppered tubes containing 5-ml tubes with 10 mM 2,4-dinitrophenylhydrazine in 1 ml of dimethylformamide and 5 μg of butyraldehyde as the internal standard. The dinitrophenylhydrazones were purified and analyzed by GC/MS (15). All incubation mixtures had a final volume of 2 ml and contained 8–50 mM 1:1 mixtures of labeled ethanol in 50 mM potassium phosphate buffer, pH 7.4. Reaction mixtures with microsomes and mitochondria contained 0.5 mM sodium azide. Unless stated otherwise, incubations with mitochondria were performed in 30 mM glycine buffer, pH 10.0, in the presence of 5 mM calcium cyanamide and 2 mM NAD^+ or 2 mM $NADP^+$. Cytosolic incubations designed for analysis of $^D(V/K)$ for ADH contained 6 mM NAD^+ and 0.5 mM azide. A similar system used for analysis of catalase contained glucose oxidase (40 units) and glucose (100 mM). Incubations designed for quantitative analysis were also carried out with semicarbazide in the inner tubes, and the absorbance of the semicarbazide solution was determined at 224 nm (*cf.* Ref. 23). All incubations were carried out at 37 °C and were performed with material corresponding to 1–7 mg of protein. Control incubations were performed by adding cofactor after trichloroacetic acid.

The $^D(V/K)$ values (24, 25) in the *in vivo* experiments were determined as described by Cronholm (19). Calculations of the $^D(V/K)$ values in the *in vitro* experiments and measurements of the stereoselectivity were performed as described by Ekström *et al.* (15).

Reduction of the [$^2H_4$]Acetaldehyde in Vitro—Incubations were carried out with either cytosol or mitochondrial fractions corresponding to 1 and 5 mg of protein, respectively. All incubations contained 5 mM [$^2H_4$]acetaldehyde in 50 mM potassium phosphate buffer, pH 7.4, 2 mM NADH, and 5 mM calcium cyanamide. [1,1-$^2H_2$]Ethanol was added as the internal standard after termination of the incubations with perchloric acid. The ethanols were analyzed as 3,5-dinitrobenzoates (19) by GC/MS.

RESULTS

In Vivo Experiments—Following intraperitoneal administration of 1:1 mixtures of [1,1-$^2H_2$]- and [2,2,2-$^2H_3$]ethanol at a dose of 2 g/kg, the rate of decrease in the concentration of ethanol as determined by GC/MS of blood samples was 50% lower in the ADH^- compared with ADH^+ animals (Table I; see Fig. 2). Reduction of acetaldehyde back to ethanol occurred at equal rates in the two strains of deer mice, as evidenced by the appearance of [$^2H_1$]- and [$^2H_4$]ethanol in the blood samples (Figs. 1 and 2). The formation of tetradeuterated molecules indicates that the coenzyme bound to the enzyme catalyzing this exchange did not exchange rapidly with unlabeled coenzyme.

TABLE I

Ethanol-acetaldehyde exchange during ethanol elimination in deer mice in vivo

The ethanol mixture consisted of [1,1-$^2H_2$]- and [2,2,2-$^2H_3$]ethanol. Values are mean ± S.D., and the numbers of animals are in parentheses.

Animals	Decrease in ethanol concentration	Rate of acetaldehyde reduction (fraction of net ethanol elimination)	Relative intermolecular hydrogen transfer	$^D(V/K)$
	mM/h		%	
ADH^+ (4)	9.6 ± 1.4	0.8 ± 0.5	64 ± 9	3.2 ± 0.8
ADH^+ + 4-MP[a] (2)	5.0; 4.2	1.3; 1.4	64; 61	2.8; 2.9
ADH^- (4)	4.7 ± 1.0	1.1 ± 0.6	73 ± 18	2.2 ± 0.1[b]
ADH^- + 4-MP[a] (2)	3.3; 3.4	2.4; 2.3	84; 79	2.2; 2.7

[a] 0.5 mmol/kg 4-MP was given intraperitoneally 15 min prior to the ethanol.

[b] $p < 0.05$ compared with ADH^+.

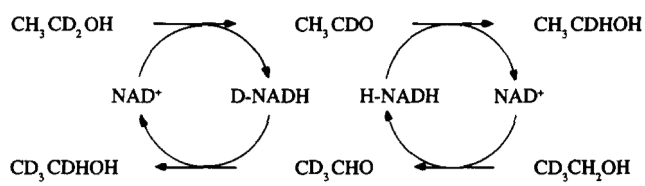

FIG. 1. **Hydrogen exchange during ethanol oxidation of a mixture of [1,1-$^2H_2$]ethanol and [2,2,2-$^2H_3$]ethanol.**

The rate of ethanol-acetaldehyde exchange was high in both ADH^- and ADH^+ deer mice as evident from the extensive rate of reduction of acetaldehyde (Table I). The distribution volumes for ethanol (mean values of 0.74 and 0.69 ml/g for ADH^+ and ADH^-, respectively) were obtained from the regression lines calculated from the decreases in ethanol concentrations. Using these volumes for calculations, the rates of net ethanol oxidation were 7.1 and 3.3 μmol/g/h, and the rates of acetaldehyde reduction were 5.2 and 3.8 μmol/g/h in ADH^+ and ADH^- deer mice, respectively.

The relative intermolecular hydrogen transfer, *i.e.* the ^2H content of the hydrogen incorporated during acetaldehyde reduction, was about 70% of that in the 1-pro-*R* position in the ethanol in both ADH^+ and ADH^- deer mice. The $^D(V/K)$ value of the ethanol oxidation was higher in the ADH^+ deer mice (3.2 ± 0.8) than in the ADH^- strain (2.2 ± 0.1) (Table I).

Pretreatment of the animals with 4-MP (0.5 mmol/kg) caused a decrease in the rate of ethanol elimination *in vivo* by about 50% in ADH^+ deer mice, but less effect was seen using ADH^- deer mice. The isotope effects, intermolecular hydrogen transfer, and rates of acetaldehyde reduction were not significantly altered by 4-MP treatment.

Liver Perfusions—By using 1:1 mixtures of [1,1-$^2H_2$]- and [2,2,2-$^2H_3$]ethanol and of [1-^{13}C]- and [1,1,2,2,2-$^2H_5$]ethanol significant formation of [1,2,2,2-$^2H_4$]ethanol or [1-^{13}C,1-^{2}H] ethanol occurred in livers from ADH^+ and ADH^- deer mice ($n = 9$), indicating that the transfer of deuterium from [1,1-$^2H_2$]ethanol to [2,2,2-$^2H_3$]ethanol or from [1,1,2,2,2-$^2H_5$] ethanol to [1-^{13}C]ethanol via a coenzyme occurred during oxidoreduction of ethanol (*cf.* Fig. 1). By using the same

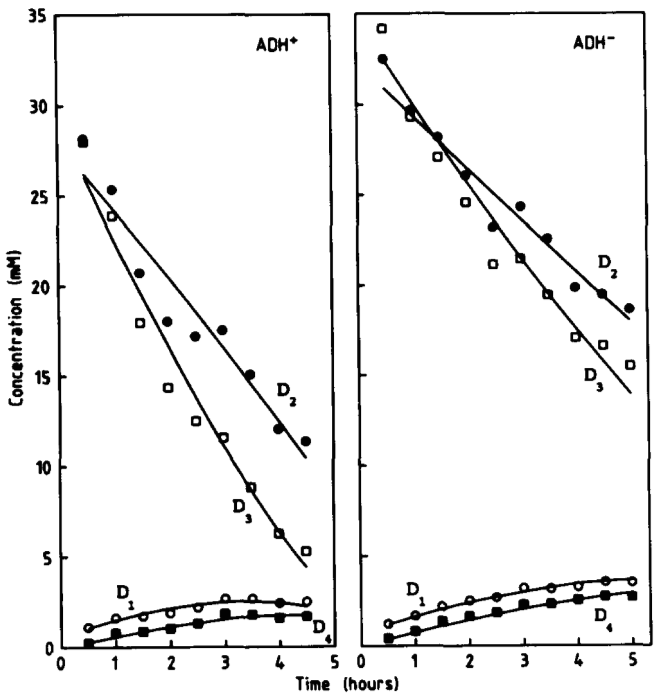

FIG. 2. **Ethanol-acetaldehyde exchange with intermolecular hydrogen transfer in deer mice *in vivo*.** A 1:1 mixture of [1,1-$^2H_2$]- and [2,2,2-$^2H_3$]ethanol (2 g/kg) was injected into ADH$^+$ and ADH$^-$ deer mice, and the concentrations of [$^2H_1$]ethanol (○, D_1), [$^2H_2$]ethanol (●, D_2), [$^2H_3$]ethanol (□, D_3), and [$^2H_4$]ethanol (■, D_4) in the blood were measured at 30-min intervals. The rate of ethanol elimination was obtained by linear regression of the concentrations. The concentrations of [^{2}H]ethanol species were calculated from the observed isotopic composition and the total concentration obtained from the linear regression. The individual concentrations of [^{2}H]ethanol species were also calculated (19) by using different values of isotope effects, relative rate of acetaldehyde reduction, and labeling of the hydrogen incorporated during this reduction. The set of values giving the smallest deviation with the least square method (given in Table I) was used to construct the curves (D_1–D_4). One experiment out of four, with similar results, is shown in the figure.

method for evaluation as applied for the *in vivo* data, it was found that the absolute rate of acetaldehyde reduction was 31 ± 10 and 24 ± 2 μmol/h (n = 3), and the relative intermolecular hydrogen transfer was 61 ± 13 and 78 ± 10% (n = 3) in the experiments with livers from ADH$^+$ and ADH$^-$ deer mice, respectively. The rate of acetaldehyde reduction in isolated liver was about 35–50% of the rate observed *in vivo*.

In Vitro Experiments—$^D(V/K)$ values of ethanol oxidation of about 3.6 were reached when incubations with liver microsomes from either deer mouse strain were performed in the presence of azide using NADPH as the cofactor. Catalase-dependent ethanol oxidation in liver cytosol exhibited a kinetic isotope effect of about 1.8, regardless of the strain, whereas ADH-dependent oxidation of the ethanol in cytosol from ADH$^+$ deer mice exhibited a $^D(V/K)$ value of about 3.2. The H_2O_2-dependent oxidation of ethanol in cytosol was stereoselective, and only the 1-pro-*R* hydrogen atom was removed from the alcohol (see Table III). By contrast, the microsomal NADPH-dependent ethanol oxidation lacked stereoselectivity (see Table III).

Mitochondria from both deer mouse strains catalyzed an NAD$^+$-dependent oxidation of ethanol with a kinetic isotope effect of 2.2. The reaction was not inhibited by 4-MP, and NADP$^+$ could not replace NAD$^+$ as the cofactor (Table II). In separate experiments[2] it was found that the NAD$^+$-dependent mitochondrial activity had a pH optimum of 10.0 and an apparent K_m for ethanol of 6.2 ± 3.0 mM. The mitochondrial ADH activity was stereoselective for removal of the 1-pro-*R* hydrogen atom in preparations from both ADH$^+$ and ADH$^-$ deer mice (Table III).

Reduction of [$^2H_4$]acetaldehyde with NADH as the cofactor occurred in cytosol (in ADH$^+$) or mitochondria from both deer mouse strains as analyzed by GC/MS of derivatized ethanol. A high rate of acetaldehyde reduction was seen in cytosol from ADH$^+$ deer mouse livers (187 ± 80 nmol/mg of protein/min (n = 3)), but acetaldehyde reduction also occurred in liver mitochondrial fractions from ADH$^+$ and ADH$^-$ deer mice at rates of 4.9 ± 0.6 and 3.2 ± 0.4 nmol/mg of protein/min (n = 3), respectively.

DISCUSSION

The results are consistent with the presence of dehydrogenase activity in ADH$^-$ deer mice that catalyzes a significant part of ethanol oxidation. This is supported by the rapid and similar rate of ethanol-acetaldehyde exchange *in vivo* in both ADH$^+$ and ADH$^-$ deer mice, which indicates the action of a reversible system. Extensive intermolecular hydrogen transfer during this exchange supports the concept that a common enzyme is responsible for oxidation and reduction and that this enzyme is a dehydrogenase. Perfusion experiments indicated that the intermolecular hydrogen transfer occurred in the liver. Furthermore, *in vitro* experiments demonstrated the presence of NAD$^+$-dependent ethanol oxidation and NADH-dependent acetaldehyde reduction in mitochondria from both ADH$^+$ and ADH$^-$ deer mice. The cofactor requirement and the stereoselectivity were the same as for cytosolic ADHs, but the $^D(V/K)$ value of 2.2 and the lack of sensitivity to 4-MP distinguish it from the cytosolic enzyme.

The $^D(V/K)$ value for ethanol elimination as determined *in vivo* in ADH$^-$ deer mice corresponded well with the isotope effects of the mitochondrial ADH activity. This value was also in agreement with that exhibited by deer mouse catalase-H_2O_2-dependent ethanol oxidation, whereas the kinetic isotope effect of hepatic cytochrome P-450-dependent ethanol oxidation was much higher. This indicates that the contribution of cytochrome P-450 to ethanol elimination in ADH$^-$ deer mice is minimal, a proposal in agreement with recent data presented by Handler *et al.* (5). Based on turnover experiments performed *in vitro*, they calculated the contribution of cytochrome P-450 to be 8% of ethanol elimination in ADH$^-$ deer mice.

The maximal contribution to ethanol oxidation of irreversible non-ADH pathways may be calculated since the rate of labeling of NADH bound to the dehydrogenase must be larger than the rate of label removal by acetaldehyde reduction and by net dehydrogenase-catalyzed oxidation. In addition, labeled NADH is lost by exchange with free less labeled NADH as seen from the incomplete intermolecular hydrogen transfer. The maximum ratio (i/d) between the rates of oxidation catalyzed by irreversible (i) and reversible (d) pathways can then be calculated from the relative intermolecular hydrogen transfer (y) and the rate of acetaldehyde reduction relative to the rate of ethanol elimination (r) obtained from Table I. With a being the labeling of ethanol in the 1-pro-*R* position divided by the isotope effect, the following expression is obtained.

$$(r + 1)da > r\,(i + d)ay + day$$

This makes it possible to calculate that the maximal ratios between irreversible and reversible pathways (i/d) equal 1.3 in the ADH$^+$ and 0.7 in the ADH$^-$ deer mice, respectively.

[2] G. Ekström, C. Norsten, T. Cronholm, and M. Ingelman-Sundberg, unpublished observations.

TABLE II

Kinetic deuterium isotope effects of ethanol oxidation in subcellular preparations from ADH$^+$ and ADH$^-$ deer mouse liver

The isotope effects were determined by GC/MS analysis. The product ratio was calculated after oxidation of a 1:1 mixture of [$^2H_6$]- and [1-^{13}C]ethanol or [1,1-$^2H_2$]- and [2,2,2-$^2H_3$]ethanol. The values are mean ± S.D., and the number of experiments is given in parentheses.

Enzyme system	Cofactor	Labeled ethanols used		Rate
		$^2H_6$ and 1-^{13}C	1,1-$^2H_2$ and 2,2,2-$^2H_3$	
				nmol/mg/min
Microsomes				
ADH$^+$	NADPH	2.8 ± 0.2 (4)	3.2 ± 0.3 (4)	7.7 ± 3.2 (6)
+ Desferrioxamine[a]	NADPH	4.0 ± 0.6 (5)	3.5 ± 0.4 (5)	6.6 ± 2.7 (5)
ADH$^-$	NADPH	3.8 ± 0.4 (4)	3.8 ± 0.5 (4)	7.8 ± 1.6 (6)
+ Desferrioxamine[a]	NADPH	4.1 ± 1.1 (4)	3.5 ± 0.8 (4)	5.9 ± 2.3 (6)
Cytosol				
ADH$^+$	H_2O_2	1.8 ± 0.2 (4)	1.9 ± 0.2 (4)	23.4 ± 9.6 (6)
ADH$^-$	H_2O_2	1.7 ± 0.3 (4)	1.9 ± 0.3 (3)	24.3 ± 9.7 (6)
ADH$^+$	NAD$^+$	3.1 ± 0.1 (3)	3.4 ± 0.4 (4)	8.4 ± 2.4 (6)
ADH$^-$	NAD$^+$	(2)	(2)	<0.1 (6)
Mitochondria				
ADH$^+$	NAD$^+$	2.2 ± 0.2 (4)	1.8 ± 0.1 (6)	3.5 ± 2.1 (11)
ADH$^-$	NAD$^+$	2.1 (2)	2.4 ± 0.5 (4)	1.6 ± 0.6 (12)
+ 4-MP[b], ADH$^-$	NAD$^+$			1.6 ± 0.3 (6)
ADH$^+$	NADP$^+$		(2)	<0.1 (2)
ADH$^-$	NADP$^+$		(2)	<0.1 (2)

[a] Concentration, 100 μM.
[b] Concentration, 0.5 mM.

TABLE III

Stereoselectivity in the oxidation of either (1R)-[1-^{2}H]ethanol or (1S)-[1-^{2}H]ethanol by subcellular systems from deer mice

The mitochondria were incubated with 1:1 mixtures of the chiral ethanol and [2,2,2-$^2H_3$]ethanol. The ratio between acetaldehydes labeled differently was measured by GC/MS. The stereoselectivity is expressed as the probability of the removal of a 1-pro-*R* hydrogen. Values are the mean ± S.D.; the numbers of experiments are given in parentheses.

Enzyme system	Stereoselectivity determined with	
	(1*R*)-[1-^{2}H]Ethanol	(1*S*)-[1-^{2}H]Ethanol
	%	
H_2O_2-cytosol		
ADH$^+$	99 ± 1 (4)	107 ± 4 (4)
ADH$^-$	100 ± 1 (4)	101 ± 2 (4)
NADPH-microsomes		
ADH$^+$	58 ± 3 (3)	41 ± 1 (3)
ADH$^-$	52 (2)	42 (2)
NAD$^+$-mitochondria		
ADH$^+$	84 ± 2 (3)	102 (2)
ADH$^-$	78 (2)	94 (2)

Thus, from this calculation we conclude that at least half of the ethanol elimination was catalyzed by the reversible pathway. The calculations depend on the assumption that the acetaldehyde formed in the reversible and irreversible pathways mixes completely. The observed oxidoreduction of ethanol in the perfusion experiments indicates that this assumption is probably valid. The rate of dehydrogenase activity in the mitochondrial fraction of the ADH$^-$ deer mouse liver is, however, too small to account for all the dehydrogenase activity observed *in vivo*. Further work is needed to elucidate the optimal conditions for this enzyme activity *in vitro*.

The conclusion regarding the absence of ADH in ADH$^-$ deer mice has been based on (i) the lack of spectrophotometrically determinable ADH activity in whole liver homogenates (6); (ii) the absence of any ADH band in starch gel electrophoretic analysis of 30,000 × *g* liver supernatants (6); and (iii) the failure of anti-ADH IgG prepared against two forms of ADH purified from ADH$^+$ deer mice to detect any ADH in livers from ADH$^-$ animals (7). We also cannot detect either reduction of NAD$^+$ in the presence of ethanol or oxidation of ethanol by homogenates from ADH$^-$ deer mice livers (not shown). The reasons are presently unknown but might be related to the presence of endogenous inhibitors. Furthermore, the structure of ADH enzymes isolated from ADH$^+$ deer mice may differ significantly from mitochondrial or other types of ADH. Therefore, antibodies prepared against these enzymes may be ineffective.

In a recent study using deer mice, Alderman *et al.* (14) concluded that the cytochrome P-450 system is the predominant catalyst of ethanol oxidation in ADH$^-$ deer mice. Their conclusion was based on calculations from determinations of *in vivo* and *in vitro* isotope effects of ethanol oxidation. The major reason for the discrepancy in comparison with our results appears to be the assumption by these workers that no reversibility of ethanol oxidation occurs in deer mice. This assumption was based on the fact that no radioactivity was found in the ethanol pool after 15 min when a trace amount of [1,2-^{14}C]acetaldehyde was added to the unlabeled ethanol injected into the deer mice. Since acetaldehyde is very reactive and only a trace amount was injected, it is likely that the radioactivity was trapped on molecules intraperitoneally or oxidized extrahepatically and thus did not equilibrate with hepatic acetaldehyde. Moreover, the isotope effect (1.4) of cytochrome P-450-dependent oxidation of ethanol as deter-

mined by Alderman *et al.* (14) is much lower than that obtained in our present or previous studies and was probably reached because it was assumed that the cytochrome P-450 system oxidizes ethanol in a stereoselective manner (*cf.* Ref. 26), which apparently is not the case (Table III and Ref. 15).

In conclusion, the determination of hydrogen exchange and kinetic isotope effects of ethanol elimination and oxidation *in vivo* and *in vitro* in ADH^+ and ADH^- deer mice suggests that dehydrogenases are important for oxidation in both strains of deer mice. Thus, oxidation of ethanol in ADH^- deer mice cannot be taken as *de facto* evidence for ethanol oxidation by non-ADH systems. Future studies are needed to quantitate more accurately the contribution of the dehydrogenase and catalase-H_2O_2 systems to ethanol elimination in deer mice.

Acknowledgments—We are indebted to Ann-Louise Hagbjörk and Britt Andrée for skillful technical assistance and to Maj-Lis Solberg for collecting blood samples.

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